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Interaction of hepatocytes and pancreatic islets cotransplanted in polymeric matrices

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Abstract Heterotopic hepatocyte transplantation (HcTx) in polymeric matrices may become an alternative to liver transplantation for metabolic disorders. Hepatotrophic stimulation by means of a portocaval shunt operation is an established, but invasive, procedure used to optimize hepatocyte engraftment in matrices. We evaluated hepatocyte and pancreatic islet cotransplantation (ICT) as an alternative noninvasive approach to hepatotrophic stimulation. Lewis rats served as donors and recipients. Hepatocytes and islets were isolated using collagenase digestion and seeded into polyvinylalcohol matrices. HcTx and ICT were compared with HcTx plus portocaval shunt and HcTx without stimulation. Matrices were investigated at 1, 3, and 6 months after implantation: the test methods applied were trichrome staining, PAS, immunohistochemistry for insulin, glucagon and incorporated BrdU, and in situ hybridization for albumin RNA. Hepatocytes expressed albumin RNA and formed conglomerates without atypias in all animals. ICT and portocaval shunting increased the number of hepatocytes and BrdU uptake. Alpha cells migrated into the islet-surrounding hepatocytes, whereas beta cells remained immobile. It is concluded that ICT and portocaval shunting supported engraftment of hepatocytes in polymeric matrices equally well. ICT did not interfere with recipient glucose metabolism and did not induce hyperproliferative premalignant foci within the transplanted hepatocytes. The technique is an attractive approach to hepatotrophic stimulation of bioartificial liver equivalents.

Key words Hepatic tissue engineering · Hepatocyte transplantation · Islet cotransplantation · Hepatotrophic stimulation

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Introduction

Liver transplantation is an established and successful procedure that represents the only causal and curative therapy for many liver diseases, such as liver cirrhosis, primary biliary cirrhosis, and tyrosinaemia [27]. Despite its therapeutic potential its application remains limited by donor organ shortage [10], and it involves the need for a life-long immunosuppressive therapy with its associated risks [5]. This situation makes the search for alternatives to whole-organ transplantation an important topic in current transplantation research.

In general, there are two different approaches. Extracorporeal systems are suitable for acute intervention in cases of fulminant hepatic failure or intoxication and may serve as a bridge to liver transplantation [7]. However, in many liver diseases in which much of the liver function is intact and organ architecture is normal, what is required is correction or replacement of one small sector of the complex of liver functions [21]; in the future, this may be accomplished by gene therapy [25]. The transplantation of a hepatocyte mass equivalent to 10% of the recipient's liver would be sufficient to normalize the metabolic situation in many enzyme deficiencies [1]. For this purpose, intracorporeal systems based on the transplantation of isolated liver cells are desirable, and liver cells have been injected in several sites [13, 20, 26]. Although first clinical trials of intraportal hepatocyte injection have been encouraging [6], limited cell numbers and portal hypertension are disadvantageous aspects of liver cell injection into preformed organs or structures [2].

Another approach to intracorporeal support is heterotopic liver cell transplantation using highly porous polymeric matrices, a form of hepatic tissue engineering [18]. Cell-seeded matrices have been implanted in several heterotopic sites. They allow the transplantation of hepatocytes up to whole-organ equivalents and can easily be removed in case of complications [32]. The production of polymeric sponges with extracellular matrix components such as collagens saturated with growth factors provides several possible ways of optimizing the microenvironment for hepatocyte engraftment [12, 23]. The aim of this concept is the creation of bioartificial, implanted liver support devices that are available without restriction. Ultimately, such devices may also be used for the implantation of genetically engineered, homologous hepatocytes in cases of metabolic enzyme deficiencies [8].

The feasibility of heterotopic hepatocyte transplantation in matrices has been demonstrated in long-term studies [17]. Nevertheless, initial engraftment rates are suboptimal. In theory, the metabolic situation in patients with hepatic failure or other liver diseases may provide a hepatotrophic stimulus for hepatocytes in heterotopic locations per se. However, such hepatotrophic effects were not observed in animal models of metabolic enzyme deficiencies [14]. Since optimal transplantation efficiency is a prerequisite for any future clinical application, improvement of engraftment and continuous long-term stimulation of hepatocytes in the polymeric matrices are of great importance. Portocaval shunt operation in the recipient is a standard procedure for experimental long-term stimulation of heterotopic hepatocytes [24], but the need for vascular surgery combined with the procedure-specific side effects [9] may reduce its applicability in humans. Selective segmental liver transplantation experiments by Starzel et al. [30] have revealed that most of the hepatotrophic factors in the portal venous blood originate from the pancreatic circulation. Therefore, pancreatic islet cotransplantation seems to be an alternative for the stimulation of hepatocytes in polymeric matrices [16].

Although hyperproliferative liver acini may be induced by intraportal islet transplantation in diabetic rats, because of excessive local concentrations of insulin and glucagon [3], the contribution of the organ-specific environment to this phenomenon is still unknown. Apart from its expected therapeutic potential, the concept of cell transplantation into porous matrices offers the possibility of studying differentiation and cellular interaction in vivo.

The object of our study was twofold: stimulation of hepatocytes in polymeric matrices by islet cotransplantation was evaluated with regard to future clinical application, and hepatocyte and islet interaction was examined on the background of the possible preneoplastic transformation of hepatocytes surrounding pancreatic islets. Hepatocytes and pancreatic islets were observed in an animal model over a period equivalent to one quarter of the recipient animals' life expectancy. Differentiation of the transplanted cell populations in the neo-tissue was investigated with morphological, morphometric and molecular biological techniques. The assessment of bromodesoxy-uridine (BrdU) uptake in vivo was used to estimate DNA synthesis and proliferation.

Materials and methods

Hepatocytes and islets were cotransplanted into highly porous polymeric polyvinyl-alcohol (PVA) matrices and compared with unstimulated hepatocytes and hepatocytes stimulated by a portocaval shunt operation. Cell-free matrices were used as controls. Intact matrices were harvested for histological examination at three time points. Matrices were investigated by trichrome staining, morphometric assessment of hepatocyte area, PAS reaction, Prussian blue staining, immunohistochemical staining for insulin, glucagon and in vivo-incorporated BrdU (cell proliferation), and in situ hybridization for albumin RNA.

Recipients were divided in four groups (Table 1). Each group contained six recipients, with a total of 24 implants. Recipients in group B underwent a portocaval shunt operation [19], while recipients in groups A, C, and D underwent a sham operation, consisting of portocaval cross-clamping for 15 min, at the same time. One week later, each recipient received four PVA matrices (Unipoint Industries, High Point, N.C.; porosity 95%, pore size 300–400 µm, diameter 12 mm, thickness 1 mm; Fig. 1A). The matrices were implanted in pockets formed of small intestinal mesenteric leaves of the recipient and fixed using Prolene 5-0 (Ethicon, Hamburg, Germany) running sutures (Fig. 1B). Each matrix was soaked with a liver cell suspension containing 1.25×10^7 vital hepatocytes (groups B and C), a cell suspension containing 1.25×10^7 hepatocytes and 500 islets (group A), or cell-free cell culture medium (group D). Two recipients per group were sacrificed at 1, 3, and 6 months after implantation, and matrices were harvested and subjected to histological and morphometric investigation.

Liver cells were isolated using a modified two-step collagenase perfusion technique first described by Seglen [28]. Initially the liver was perfused via the portal vein with a calcium-free buffer solution. After 7 min it was perfused with a collagenase solution (0.5 mg/ml Collagenase H, Boehringer Mannheim, Mannheim, Germany) at 37°C for 6–8 min. The digested liver was resected, and cells were released by gentle shaking and collected in William's medium E (Gibco BRL, Eggenstein, Germany). Cells were filtered using a 200-µm nylon mesh and washed three times with William's medium E. Hepatocyte viability exceeded 85% as assessed by the tryphan blue exclusion method. Cell counts ranged from 3.0×10^8 to 4.0×10^8 hepatocytes per perfused liver.

Pancreatic islets were isolated using an intraductal collagenase injection technique [29]. Briefly, after ligation of the duodenal papilla the pancreas was injected via the bile duct with 6 ml ice-cold collagenase solution [2 mg/ml collagenase (PanPlus, Serva, Heidelberg, Germany) in M199 medium (Gibco BRL)].

Table 1 Experimental design (*ShamOP* sham operation [portocaval crossclamping], *PCS* portocaval shunt operation, *Mi* implantation of four polyvinyl alcohol matrices, *HcTx* transplantation of 1.25×10^7 vital hepatocytes per matrix, *IsCoTx* cotransplantation of 500 islets per matrix, *Explant* Explantation of matrices and morphometric and morphological evaluation)

	Group A	Group B	Group C	Group D
Day –7	ShamOp	PCS	ShamOp	ShamOp
Day 0	Mi; HcTx; IsCoTx	Mi, HcTx	Mi, HcTx	Mi
Day 30	Explant	Explant	Explant	Explant
Day 90	Explant	Explant	Explant	Explant
Day 180	Explant	Explant	Explant	Explant

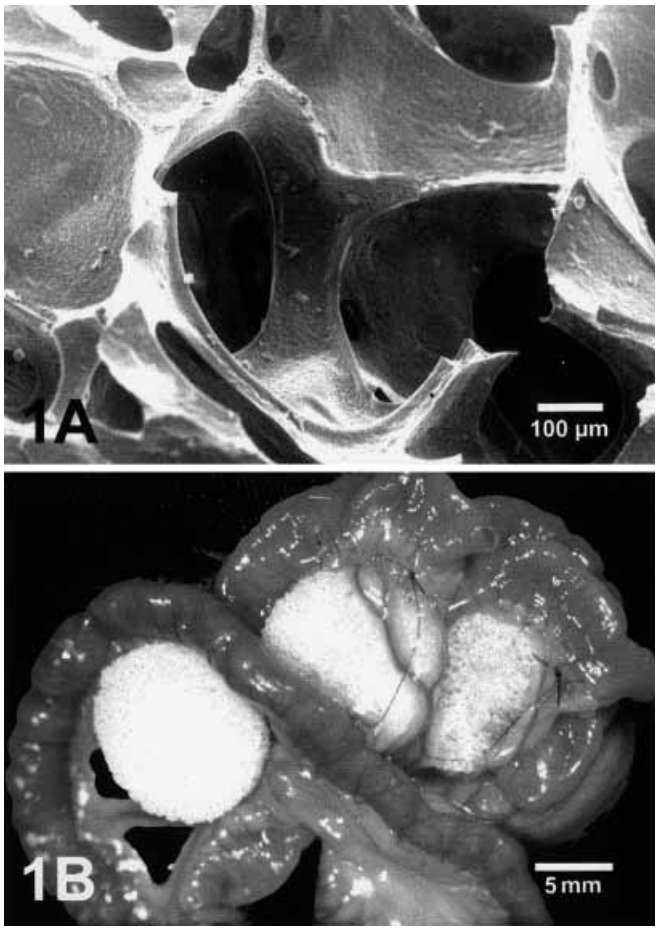


Fig. 1 **A** Scanning electron microphotograph of a highly porous polyvinyl-alcohol matrix before cell seeding. **B** Operation situs at polymer implantation. The PVA matrices are located in a pocket formed of small intestinal mesenteries and fixed with a running Prolene suture

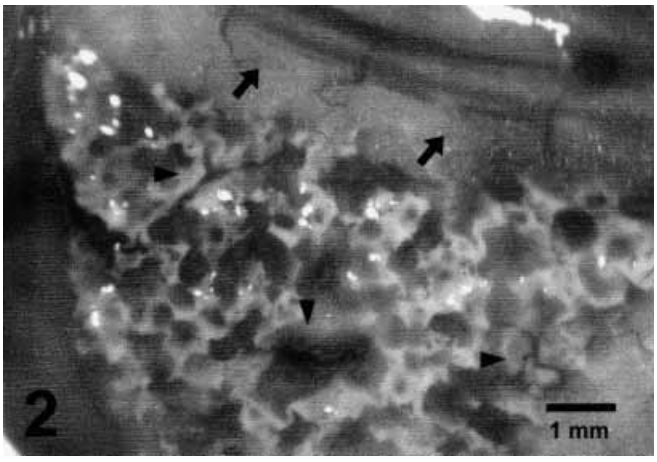


Fig. 2 Detail of a polyvinyl alcohol matrix in situ 6 months after implantation. The matrix is completely covered by smooth mesenteric tissue. The pores of the matrix are infiltrated by blood vessels with different calibres (*arrowheads*) originating from the mesenteric vessels (*arrows*)

The pancreas and the adjacent mesenteries were resected and incubated for 15 min at 37°C. After gentle shaking the dissociated islets were separated from the acinar tissue using a gradient purification (Histopaque 1077, Sigma, St. Louis, Mo., USA) followed by three washing steps with M199. For each pancreas, 600-1000 islets (diameter 100–400 µm) were obtained. Islets were cultured in M199 containing 5% fetal calf serum (Biochrom, Berlin, Germany) at 37°C, 5% CO2 for 18–24 h prior to seeding.

For cell seeding, PVA polymers were washed and prepared according to the manufacturer's recommendations. Pancreatic islets were seeded prior to hepatocytes (group A). Islets were allowed to attach to the matrices for 4 h in the incubator. Then, matrices were soaked with 250 µl of a freshly prepared liver cell suspension (groups A–C) or 250 ml cell-free William's medium E (group D). Seeding efficiency was at least 85%. Matrices stayed on ice and were implanted within 1 h.

Syngeneic male Lewis rats were used as donor and recipient animals. The animals were housed at the veterinary care facility of the University of Hamburg Medical Centre, submitted to a 12-h day/night cycle and had free access to water and standard rat chow. Surgical procedures were performed under ether inhalation anaesthesia. Recipients received one preoperative dose of antibiotics s.c. (Spizef 100 mg/kg body weight, Takeda Pharma, Aachen, Germany). German regulations for the care and use of laboratory animals were observed at all times. Recipients were injected with BrdU cell proliferation labelling reagent (20 ml/kg body weight, Amersham, Braunschweig, Germany) i.p. 6 h before sacrifice. Blood glucose levels were measured at sacrifice.

Polymers were fixed in Bouin's solution and embedded in paraffin. Cross sections 5 µm thick were obtained from the centre region of each matrix and used for histological investigations.

The area covered by hepatocytes was assessed using a computer-assisted image-analysing system (Lucia M, Nikon, Düsseldorf, Germany). Four trichrome-stained cross sections from standardized, central planes of each implant were analyzed, and the mean hepatocyte area for each matrix was calculated. These mean values were used to calculate mean hepatocyte areas for each group and time-point. This method yields results that are representative for the hepatocyte mass in the whole matrix [24]. Results are expressed as mean hepatocyte area (μm^2) ± standard deviation per 17 mm^2 total standard cross section area.

Hepatocyte proliferation ratio was assessed in BrdU-stained cross sections of four polymers per group and time and in four standard liver sections from animals in group D, 6 months after implantation. Mean proliferation ratios were calculated for each group and time. Values are given as mean proliferation ratio (%) ± standard deviation.

Statistical significance testing was performed using Student's *t*-test and Wilcoxon's nonparametric test. The *P*-values given are two-tailed. *P*-values <0.05 were considered significant.

Immunohistochemical analysis was performed as described elsewhere [31], with polyclonal antibodies specific for insulin [guinea pig anti-swine insulin, Dako, Glostrup, Denmark; 1:100 in phosphate-buffered saline (PBS)] and glucagon (rabbit anti-swine glucagon, Dako; 1:2000 in PBS) and monoclonal antibodies specific for BrdU (mouse anti-bromodesoxy Uridine, Amersham; 1:600 in PBS). Horseradish peroxidase-conjugated antibodies (rabbit anti-guinea pig, Dako; 1:600 in PBS) were applied for insulin staining, monoclonal mouse anti-rabbit (Dako; 1:200 in PBS) and rabbit anti-mouse (Dako; 1:25 in PBS+10% rat serum) antibodies, and monoclonal mouse alkaline phosphatase anti alkaline phosphatase complex (APAAP, Dako; 1:20 in PBS) were applied for glucagon and BrdU staining.

In situ hybridization was performed as recently described [11]. A 550-bp fragment encoding the carboxyterminal region of the rat serum albumin gene was subcloned into the plasmid pGEM1 (Promega Biotech, Heidelberg, Germany). After linearization of the plasmid with either EcoRl or Xbal restriction endonucleases, Sp6 or T7 RNA polymerases were used to obtain run-off transcripts of either the antisense or the sense strands in the presence of [^{35}S]uridine (Amersham). Following hybridization and washing, bound probes were detected by autoradiography.

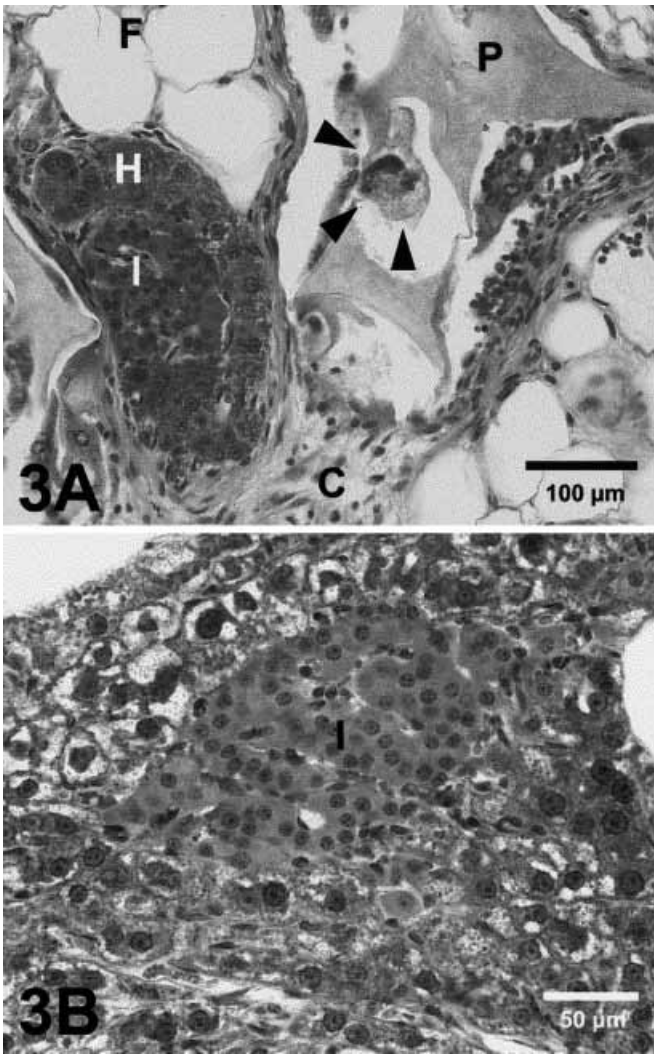


Fig. 3 Trichrome-stained matrices from group A (islet cotransplantation) at **A** 1 month and **B** 6 months after implantation. Capillarized pancreatic islets (*I*) surrounded by several layers of hepatocytes (*H*) are embedded in vascularized connective (*C*) and fatty (*F*) tissue. Multinucleated giant cells (*arrowheads*) are seen in the vicinity of the polymer (*P*). Hepatocytes with well-defined nuclei form lobated conglomerates. At 6 months some hepatocytes in close contact with pancreatic islets display vacuolized cytoplasm ***B***

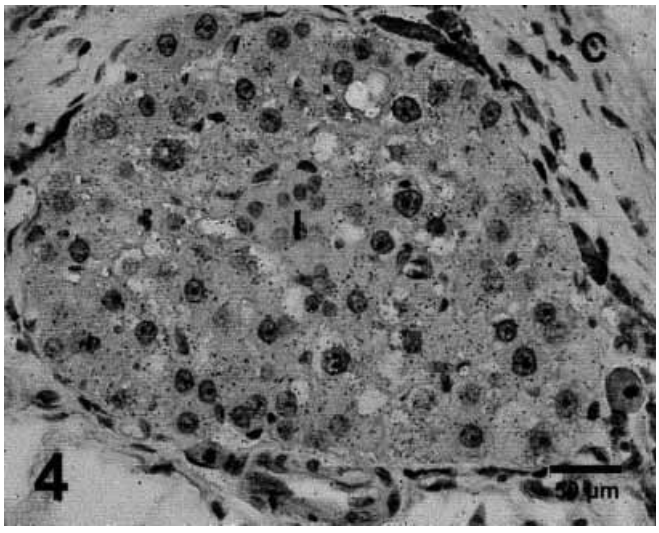


Fig. 4 Albumin expression of hepatocytes from group A (islet cotransplantation) 6 months after implantation. A strong autoradiographic signal is restricted to the hepatocytes and absent from the surrounding connective tissue (*C*) and the islet (*I*). Exposure time 14 days

Results

The surgical procedures were well tolerated. All recipients survived until their designated sacrifice points. Apart from a slightly decreased body weight of the animals in group B (portocaval shunt), no abnormalities in the appearance or behaviour of the recipients were observed. There were neither intra-abdominal adhesions nor any other macroscopically conspicuous intra-abdominal changes. The matrices appeared macroscopically intact and well vascularized (Fig. 2). Vascularization from extramesenteric blood vessels was not observed. There were no significant changes in shape, size or volume of the explanted matrices compared with matrices prior to implantation. Blood glucose levels were normal in each group at each time (Table 2).

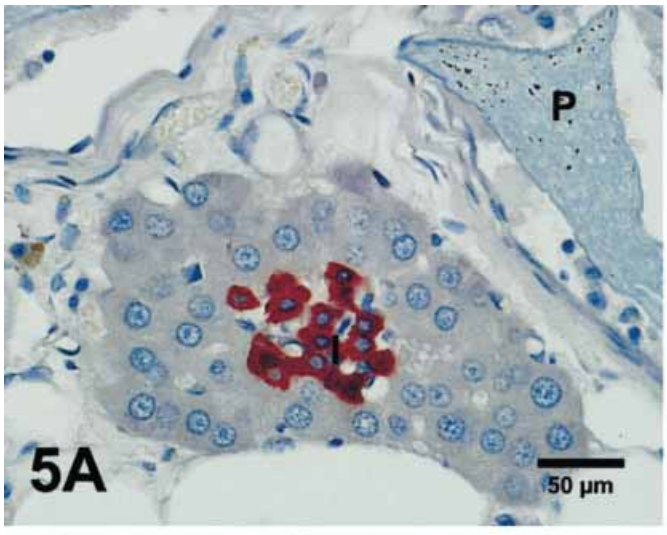
Matrices from the control group (group D, implantation of cell-free matrices) displayed infiltration by a highly capillarized fibrovascular tissue. Over the course of the experiment the connective tissue was largely replaced by fat cells. Multinucleated giant cells in close contact to the matrix were present in similar numbers at all three timepoints. At 6 months after implantation some macrophages within the connective tissue contained brownish pigment (Fig. 6, left lower corner) that was identified as haemosiderin by Prussian blue staining. Neither hepatocytes, as evidenced by morphology and albumin RNA expression, nor insulin- or glucagon-positive islet cells were observed in matrices from the control group.

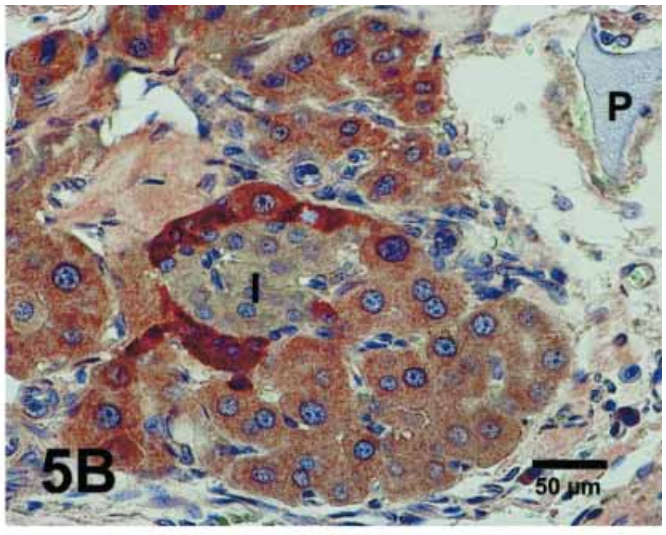
The composition of the nonparenchymal tissue in matrices from the cell transplantation groups (groups A–C) was similar to that in the control group (group D). In these matrices clusters of hepatocytes with large, spheri-

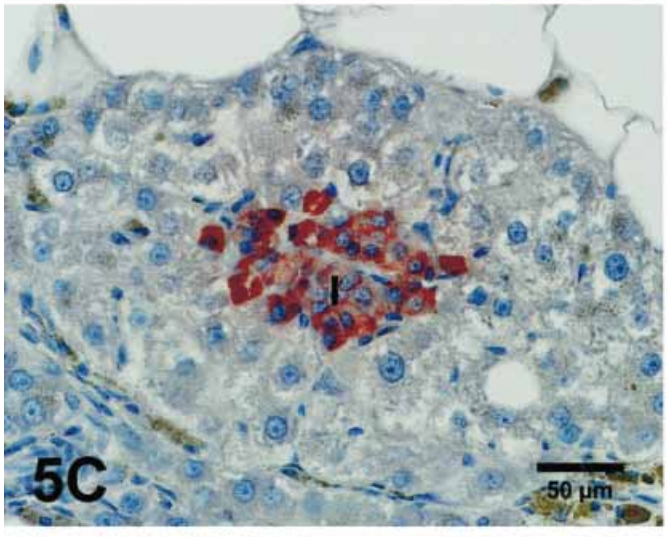
Fig. 5 Cotransplanted pancreatic islets (*I*) 1 month after transplantation with **A** insulin-positive beta cells in the center and **B** glucagon-positive alpha cells at the periphery. Islets are surrounded by hepatocytes, connective tissue, and polymer (*P*). **C** Beta cells remain immobile, while **D** alpha cells migrate into the surrounding hepatocytes 6 months after implantation. **A, C** Insulin staining using the HRP technique, **B, D** glucagon staining using the APAAP technique

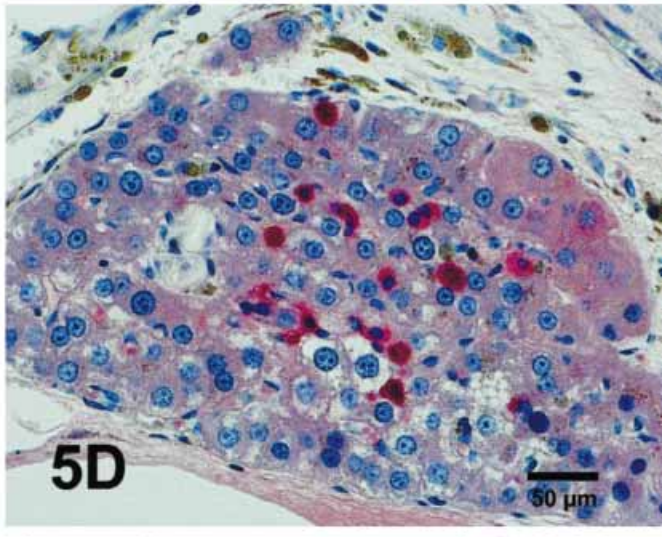
Fig. 6 BrdU immunohistochemistry demonstrates active DNA synthesis in one hepatocyte (*arrow*) in a matrix from group A 6 months after implantation. Hepatocytes in this group do not display any abnormal proliferative activity. Hepatocytes are surrounded by connective tissue with some proliferating fibroblasts and macrophages. Some macrophages in the hepatocyte-surrounding connective tissue contain brownish hemosiderin pigment (*lower left corner*). BrdU injection 6 h before sacrifice

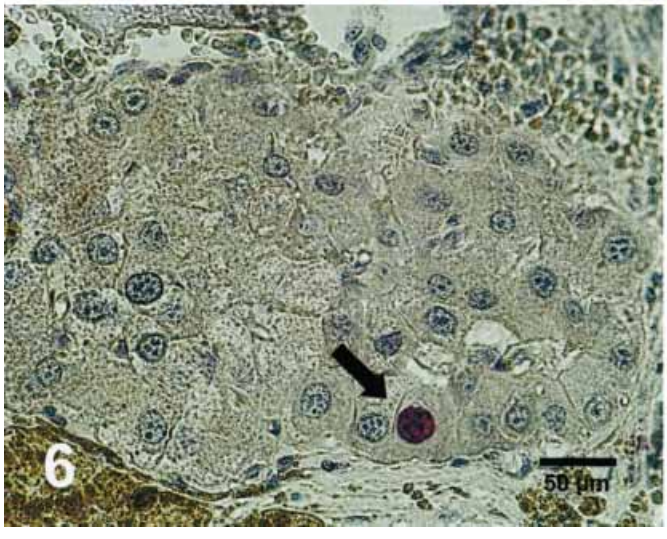
Fig. 7 PAS staining of hepatocytes stimulated by islet cotransplantation 6 months after transplantation. Stimulated hepatocytes display an inhomogenous and increased PAS activity

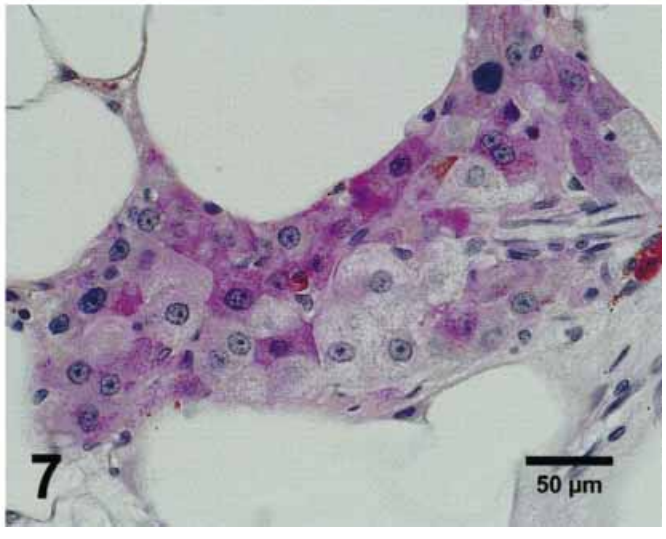












cal and well-defined nuclei were embedded in capillarized connective tissue (Fig. 3A,B). Hepatocytes had no direct contact with the polymer. Between 1 and 6 months after implantation, hepatocytes became more cuboidal and formed capillarized conglomerates 200–500 µm in diameter. Hepatocyte conglomerates in group C (hepatocyte transplantation in unstimulated matrices) were apparently smaller than in group A (hepatocyte and islet cotransplantation) and group B (hepatocytes transplanted in recipients with portocaval shunt operation prior to hepa-

Table 2 Blood glucose levels of the recipients at the time of explantation

	Group A	Group B	Group C	Group D
Day 30	111 mg/dl 124 mg/dl	83 mg/dl 89 mg/dl	115 mg/dl 112 mg/dl	108 mg/dl 120 mg/dl
Day 90	87 mg/dl 96 mg/dl	80 mg/dl 86 mg/dl	103 mg/dl 96 mg/dl	100 mg/dl 105 mg/dl
Day 180	112 mg/dl 101 mg/dl	66 mg/dl 75 mg/dl	80 mg/dl 82 mg/dl	90 mg/dl 98 mg/dl

tocyte transplantation). Initially, PAS reaction in hepatocytes from all groups was slightly decreased. However, at 3 and 6 months after implantation it normalized in groups B (portocaval shunt) and C (unstimulated). Hepatocytes in group A (islet cotransplantation) displayed increased PAS activity with inhomogeneous distribution (Fig. 7) at this time. Independent of hepatotrophic stimulation, hepatocytes displayed normal albumin RNA transcript levels in all cell transplantation groups at all times, comparable to those in normal rat liver (Fig. 4). Anticomplementary (sense) probe controls demonstrated hybridization specificity. BrdU-positive proliferating hepatocytes were found in every matrix from groups A–C (Fig. 6).

Intact, capillarized islets with beta cells in the centre and alpha cells at the periphery were observed in group A (islet cotransplantation) 1 month after implantation (Fig. 5A,B). Every pancreatic islet is surrounded by several layers of hepatocytes. Between 1 and 3 months, alpha cells started to migrate into the surrounding hepatocytes. Beta cells maintained contact with the islet structure at this time. But 6 months after implantation, virtually all alpha cells had lost contact with the remaining beta cells. However, no alpha cells were seen beyond the surrounding hepatocyte layers (Fig. 5C,D). The intensity of insulin and glucagon staining was comparable to that in pancreatic control sections from untreated animals at all time-points. Hepatocytes in close spatial relationship to pancreatic islets did not express abnormal proliferative activity as evidenced by BrdU uptake (Fig. 6). Apart from slightly vacuolized cytoplasm and irregular glycogen accumulation no morphological changes in transplanted hepatocytes close to cotransplanted pancreatic islets were noticeable (Figs. 3B, 7). Albumin RNA expression was unaffected in the islet-surrounding hepatocytes (Fig. 4).

One month after implantation, the mean hepatocyte area (Fig. 8A) in group A (islet cotransplantation) and group B (portocaval shunt) was significantly (P<0.05) larger than that in the unstimulated group, C. By 3 and 6 months after implantation mean hepatocyte areas in the stimulated groups (A and B) were also larger than in the unstimulated group (C). For the portocaval shunt group (B) this difference was statistically significant at both time-points (P<0.001). The difference between the islet cotransplantation group (A) and the unstimulated group (C) was significant only at 6 months after implantation (P<0.05). Hepatocyte area increased in matrices from all

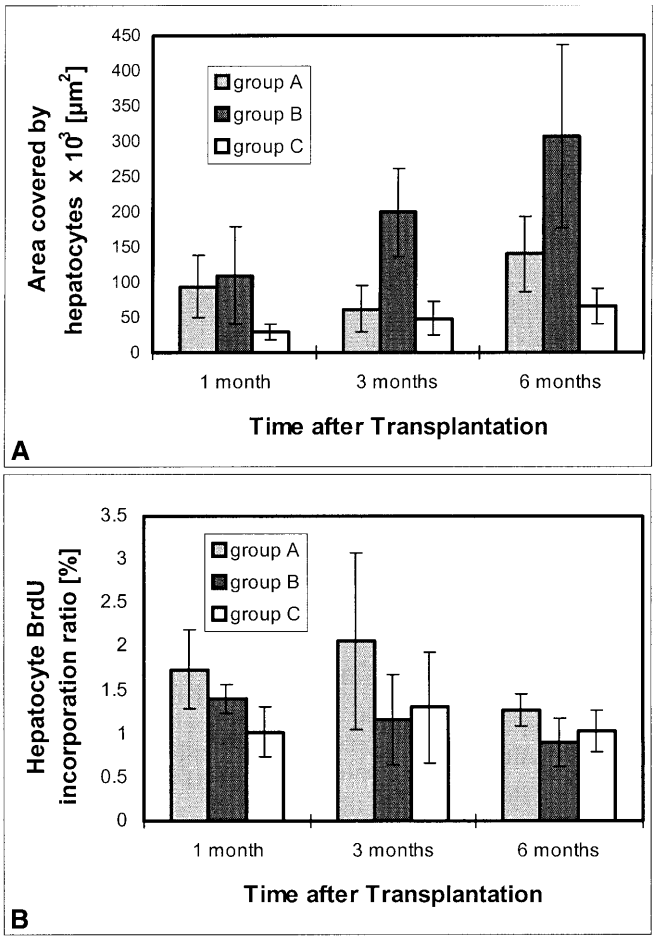


Fig. 8 Evaluation of **A** hepatocyte area and **B** hepatocyte BrdU incorporation ratio. Group A, hepatocyte transplantation (HcTx) and cotransplantation of 500 islets per matrix; group B, HcTx after stimulation by portocaval shunt operation; group C, HcTx. Each *column* represents two animals with a total number of eight matrices. Values are given as **A** mean [µm²] ± standard deviation or **B** mean [%] ± standard deviation

groups over the course of the experiment. However, this increase was only significant in groups B and C (P<0.01).

At 1 month after implantation, the hepatocyte proliferation ratios in groups A (islet cotransplantation) and B (portocaval shunt) were not significantly (P=0.056) higher than in group C (unstimulated hepatocytes; Fig. 8B). Although hepatocyte proliferation ratios seemed to decline in both stimulated groups over time, the difference observed was not significant. In contrast to groups A and B, in the unstimulated control group C the hepatocyte proliferation ratio was relatively constant within the first 6 months following implantation. At 6 months after transplantation, proliferation ratios in all three groups approached a common level of approximately 1%. The proportion of binucleated hepatocytes was below 1%. Hepatocyte proliferation ratio in the standard liver sections from group D was 0.25±0.06%. The difference between the experimental groups and the liver standard section was significant at all times (P<0.05).

Discussion

These results demonstrate the practicability of hepatocyte and islet cotransplantation in porous matrices. The existence of conglomerates of differentiated hepatocytes in close contact with well-vascularized connective tissue suggests that the matrices represent an adequate environment to support transplanted hepatocytes.

Transplanted hepatocytes maintained differentiation independently of hepatotrophic stimulation as demonstrated by normal morphology, PAS reaction and albumin RNA expression. Reduced mean size of hepatocyte conglomerates in unstimulated matrices and slightly vacuolized cytoplasm of islet-surrounding hepatocytes were the only morphologically visible difference distinguishing matrices from stimulated and unstimulated groups. Morphometric analysis disclosed the effects of hepatotrophic stimulation on heterotopically transplanted hepatocytes: hepatotrophic stimulation either by portocaval shunt operation or by islet cotransplantation significantly extended the area covered by hepatocytes in the matrices at 1 and 6 months after transplantation compared with unstimulated matrices. Hepatocyte areas in unstimulated matrices from group C and in matrices stimulated by portocaval shunt operation increased significantly between 1 and 6 months after implantation, whereas the hepatocyte area in matrices stimulated by the cotransplantation of pancreatic islets remained stable throughout the course of the experiment. This different kinetics suggests a decreased hepatotrophic stimulus of the cotransplanted islets at the end of the experiment and raises questions about the long-term efficiency of the approach presented. The observed increase in hepatocyte area in unstimulated matrices underlines the proliferative potential of hepatocytes in three-dimensional matrices even without hepatotrophic stimulation. The influence of hepatotrophic stimulation on the proliferation ratio of the transplanted hepatocytes seemed to decline within the first 3 months after transplantation. Hepatocyte proliferation was not only influenced by hepatotrophic stimulation but also by the matrix itself. Hepatocyte proliferation ratio in the matrices was at least 4 times higher than in the standard liver sections. To complete the picture, it must be mentioned that in vivo uptake of BrdU is not specific for cell proliferation; uptake can also reflect polyploidization of the hepatocyte nuclei. However, we regard BrdU uptake primarily as a marker for hepatocyte proliferation, because occasional mitotic figures were observed and the percentage of binucleated hepatocytes was below 1%. Although proliferation ratios of hepatocytes in native liver and in matrices could not be compared without reservation, the considerable difference implies that the matrix and the foreign body reaction within the matrix produce a situation of accelerated hepatocyte proliferation and hepatocyte death. Against this background, the major effect of hepatotrophic stimulation is probably a slowdown of elevated hepatocyte turnover in the matrices with a consequent rise in the number of hepatocytes over time, rather than an induction of hepatocyte proliferation alone. This is in contrast to findings of increased hepatocyte turnover in hyperproliferative hepatic foci induced by intraportal islet injection in diabetic rats [3]. The different response of hepatocytes in matrices and hepatocytes in native livers to hepatotrophic stimuli may be explained by the different initial proliferation levels and by the complex regulation of hepatocyte proliferation in the intact liver. In this experiment, the increased cell turnover in the matrices had no obvious negative influence on hepatocyte differentiation. However, the reduction of hepatocyte turnover to more physiological levels seems to be preferable. This might be achieved by using polymers with improved biocompatibility.

Cotransplanted pancreatic islets did not interfere with the recipients' glucose metabolism, as indicated by normal blood glucose levels. The cotransplanted islets are likely to contribute to the secretion of pancreas-derived hormones in the nondiabetic recipient. Assuming that the demand for these factors is unchanged after islet cotransplantation, the down-regulation of both transplanted and recipients' own islets is conceivable. Functional inactivity of the transplanted islets owing to the loss of glucose recognition structures of the beta cells is another possible explanation for the normoglycaemia throughout the entire observation period and for the decreased hepatotrophic potency of the islets at the end of the observation period. However, the intensity of insulin and glucagon staining was normal compared with standard pancreas sections from untreated animals. At 1 month after transplantation, islets appeared histologically inconspicuous. The subsequent migration of alpha cells raises questions about the structural integrity of the transplanted islets. The loss of alpha cells may also be observed after duct ligation and even in normal rat pancreata as an age-related phenomenon. However, in the matrices this phenomenon occurred as soon as 3 months after implantation. It might be a sign of premature ageing of the cotransplanted islets as a consequence of matrix-related processes, such as the evident foreign-body reaction in the polymers. The process of alpha cell migration must be investigated in future long-term studies, since further dispersal of the islets may endanger their long-term function and engraftment.

Dombrowski et al. report that the intrahepatic transplantation of islets in low numbers in diabetic recipients leads to the induction of altered liver acini and hepatocellular carcinomas [3, 4]. Preneoplastic foci have been observed within the first month after islet implantation. Although islets were implanted into nondiabetic recipients in our experiments, the completely different environment and the very high islet-to-hepatocyte ratio meant we could not exclude the possibility of induction of preneoplastic hepatocyte alterations at the beginning of the experiment. PAS reaction and BrdU immunohistochemistry did not reveal hepatocytes in contact with pancreatic islets with relevant morphological changes over a period of 6 months. It is possible that the secretory activity of the transplanted islets in the nondiabetic recipients was insufficient to induce such far-reaching changes in the hepatocyte phenotype.

In this experiment the polyvinyl-alcohol matrix provided advantageous conditions for hepatocyte engraftment. However, multinucleated giant cells, a fibroplastic reac-

tion and haemolysis with siderosis within the matrix indicate that the biocompatibility of the polymer could be improved. Since an initial ingrowth of granulation tissue is mandatory for optimal vascularization, entirely inert materials would be disadvantageous [33]. We used nondegradable polymers to achieve precise morphometric data without any changes in matrix shape or volume due to biodegradation. Biodegradable polymers such as poly-*l*-lactic acid (PLLA) and poly-glycolic acid (PGA) are, however, promising candidate matrices for future studies [22].

In conclusion, hepatocyte and islet cotransplantation in porous polymeric matrices is a feasible step towards bioartificial, implantable liver support devices. We observed no significant changes in hepatocyte morphology that might be related to cotransplantation of pancreatic islets in polymeric matrices over a period of 6 months. Islet cotransplantation increased transplantation efficiency to a desirable extent. It may facilitate continuous in vitro [15] and in vivo stimulation for the application of stable transfected, homologous hepatocytes in cases of metabolic enzyme deficiencies.

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